### Lipid Isomer Separation Using Travelling Wave Cyclic Ion Mobility Mass Spectrometry

Giorgis Isaac, Hernando Olivos, and Robert S. Plumb; Waters Corporation, Milford, MA

#### Introduction

- Galactosylceramide (GalCer) and glucosylceramide (GlcCer) are isomers and only differ in the position of the hydroxy group at the C-4 (Figure 1).
- Ganglioside GD1a and GD1b differ in the composition and sequence of sialic acid (Figure 1).
- A slight difference in chemical composition and molecular conformation contribute to profound differences in their physicochemical properties and biological functions.
- Here we Therefore, it is very important to separate these isomers to understand their biological role and function.
- Demonstrate the complete separation of these lipid isomers only possible with the multi-pass capability of the SELECT SERIES<sup>™</sup> Cyclic<sup>™</sup> ion mobility spectrometer (cIMS).



Figure 1. Chemical structure of the analyzed lipid isomers.

## **Experimental**

- GalCer d18:1/18:0, GlcCer d18:1/18:0, ganglioside GD1a (d18:1/18:0) and GD1b (d18:1/18:0) were purchased from Avanti Polar Lipids and a final concentration of 1ng/µL was prepared. Samples were infused at 5µL/min into the ESI source of the cIMS.
- Different adduct ions were selected in the quadrupole and transferred to the cyclic mobility cell for multiple passes.

### Results

**GalCer and GlcCer Isomer Separation** 



**Figure 2.** Arrival Time Distribution for the separation of GalCer (d18:1/18:0) and GlcCer (d18:1/18:0) [M-H]<sup>-</sup> m/z 726.5440 mixtures using 1(A), 5(B), 10(C), and 20(D) passes of the ion mobility device.



**Figure 3.** Arrival Time Distribution for the separation of individual GalCer (A), GlcCer (B) or the equimolar mixture of the two ceramides (C) using 20 passes of the ion mobility device.



#### Results

#### Ganglioside GD1a and GD1b Isomer Separation



**Figure 4.** Arrival time distribution for the separation of GD1a (d18:1/18:0) and GD1b (d18:1/18:0) at m/z 917.488 [M-2H]<sup>-2</sup> mixtures using (A) 1 pass, (B) 2 passes, (C) 3 passes, (D) 4 passes, and (E) 5 passes of the ion mobility device.



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**Figure 5.** Arrival time distribution for the separation of individual (A) GD1a (d18:1/18:0), (B) GD1b (d18:1/18:0), or (C) the equimolar mixture of the two ganglioside isomers at m/z 917.488 [M-2H]<sup>-2</sup> using five passes of the ion mobility device.

# Conclusions

- The cIMS has a unique multi-pass cyclic ion mobility capability, to scale ion mobility resolution to meet a given challenge.
- The GalCer (d18:1/18:0) and GlcCer (d18:1/18:0) isomers were base line resolved using twenty passes of the IM cell, with IMS resolution of 290  $\Omega/\Delta\Omega$ .
- The ganglioside isomers GD1a (d18:1/18:0) and GD1b (d18:1/18:0) were successfully resolved using 5 passes of the IM cell with IMS resolution of 145 Ω/ΔΩ.
- The scalable increased ion mobility resolution is useful to separate lipid isomers

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